

## StatLab Quantum HD 2 Step Polymer HRP Kit (Mouse/Rabbit)

**Catalog No(s):** QHD-U2-100-HRP-Kit; QHD-U2-15-HRP-Kit; SAM-QHD-U2-15-HRP  
**Format:** Ready to Use  
**Document No:** QHD-U2-15-HRP-Kit 05302018  
**Effective Date** 05/30/2018, IFU-266 Rev A

**Intended Use** For In Vitro Diagnostic Use

The Quantum HD 2 step Polymer HRP Kit is a non-biotin, two-step detection system suitable for detecting antigens in formalin-fixed paraffin-embedded tissues and cryostat sections by immunohistochemistry. It may also be used with blood smears, cytospins, and cell preparations.

The Quantum HD 2 step Polymer HRP detection kits utilize secondary antibodies labeled with horseradish peroxidase. The Quantum HD 2 Step Polymer HRP kit can be used for manual immunohistochemistry as well as any automated IHC stainer operating as an open platform.

The Quantum HD HRP polymer is a two-step detection system consisting of polymer enhancer and HRP Polymer-2. It recognizes mouse and rabbit immunoglobulins, and it detects any tissue-bound primary antibody. Sections are further incubated with the substrate/chromogen, 3,3'-diaminobenzidine (DAB) causing a reaction with the peroxidase producing a visible brown precipitate at the antigen site. Sections are counterstained with Hematoxylin. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with an antigen.

### Introduction

Optimal immunostaining not only depends on the specificity of the primary antibody and other immunoreagents but also depends on obtaining a good signal to noise ratio. Binding of an antibody to its epitopes involves van der Waals forces, electrostatic forces and hydrophobic forces. Certain antibodies have tendency to bind loosely and nonspecifically to unrelated epitopes, which can create undesired background staining. In order to remove these nonspecifically bound antibodies, a thorough washing is required after each immunostaining step. Quantum HD Immuno Wash Buffer specifically designed to remove such loosely bound antibodies effectively and efficiently and to provide a cleaner background staining.

### Storage

Store at 2 - 8°C. Do not freeze. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

### Kit Contents

| Component                     | QHD-U2-100-HRP-Kit | SAM-QHD-U2-15 HRP-Kit & QHD-U2-15-HRP-Kit |
|-------------------------------|--------------------|-------------------------------------------|
| Quantum HD Peroxidase Block   | 100 mL             | 15 mL                                     |
| Quantum HD Polymer Enhancer   | 100 mL             | 15 mL                                     |
| Quantum HD HRP Polymer-2      | 100 mL             | 15 mL                                     |
| Quantum HD DAB Substrate (2x) | 50 mL              | 7.5 mL                                    |
| Quantum HD Chromogen (2x)     | 50 mL.             | 7.5 mL                                    |
| Quantum HD Hematoxylin        | 100 mL.            | 15 mL                                     |

### Composition

All reagent components are formulated without azide or thimerosal preservatives. The reagents are provided in ready-to-use form, except for Quantum HD DAB Chromogen. SDS is available upon request.

### Material Required but Not Provided

1. Xylene or dewaxing reagents
2. Absolute ethanol
3. Distilled or deionized water
4. QHD Immuno Wash Buffer -diluted 1 portion of wash buffer to 19 parts of distilled or deionized water. #QHD-015



5. Primary Antibody Diluent- (if required)
6. Coverslips and mounting media

**Preparation of Stable  
DAB/Plus Substrate  
Working Solution**

1. Transfer 1 part of Quantum HD DAB Substrate to a tube or mixing bottle.
2. Add 1 part of Quantum HD DAB Chromogen to the 1 part of Quantum HD DAB Substrate.
3. The substrate working solution is stable for 24 hours refrigerated at 2-8°C.
4. Working solution volume can be scaled up using the same ratio of buffer to chromogen.
5. Dispose of unused DAB solutions in appropriate waste stream, according to local, state or federal regulations.

**Precautions**

1. DAB has been classified as a suspected carcinogen and can cause skin irritation upon contact. Wear personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
2. Consult local and/or state authorities regarding recommended method of disposal
3. Microbial contamination could produce erroneous results
4. Do not ingest or inhale any reagents. Reagents may cause irritation, avoid contact with eyes and mucous membranes. If reagents contact these areas, rinse with copious amounts of water. Wear disposable gloves when handling reagents.
5. There is no expressed or implied warranties which extend beyond this datasheet. StatLab is not liable for personal injury, property damage or economic loss caused by this product.
6. Do not use after expiration date stated on the vial. The user must validate any storage conditions other than those specified in the package insert.
7. Specimens, before and after fixation and all materials exposed to them, should be handled as if capable of transmitting infections and disposed of with proper precautions.

**Recommended Staining  
Protocol**

1. Paraffin embedded tissue sections must be deparaffinized with xylene or dewaxing agent and rehydrated with a graded series of ethanol and water washes before staining. Follow the standard dewaxing and rehydration protocol used in your lab.
2. The investigator needs to optimize the dilution and incubation times for primary antibodies.
3. Each immunostaining run should include known positive and negative controls to assure proper functioning of the staining system and aid in valid interpretation of the results.

Typical controls:

Positive Control: A tissue known to contain the desired antigen, which has yielded positive staining in the past.

Negative Controls:

Reagent Controls

- A. Substitute normal non-immune serum from the same host animal as the primary antibody (e.g. if using mouse monoclonal primary antibodies, use mouse non-immune serum).
- B. Substitute matching host species isotype control for primary antibody
- C. Use antigen-adsorbed primary antibody (i.e. antibody reagent which has been adsorbed with the target antigen to remove specific antibody)

Negative Tissue control – A tissue known to *not* contain the desired antigen.

4. Consult the primary antibody supplier for recommended for antigen recovery treatments. Perform epitope recovery pretreatments before starting the staining procedure.
5. Once the slide treatment has been started, DO NOT let tissues or specimens dry. This can cause undesirable background or artifacts.

**MANUAL STAINING PROCEDURE**

**INCUBATION TIME**

|                                                    |                                                                                                                                                                                                                                                                           |                                            |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| <b>1. Perform antigen retrieval (if necessary)</b> | Refer to Antibody Data Sheet for recommendations                                                                                                                                                                                                                          | Refer to Antibody Data Sheet               |
| <b>2. Quantum HD Peroxidase</b>                    | A. Incubate slides in Quantum HD Peroxidase Block.<br>B. Rinse slides with QHD Immuno Wash Buffer three (3) times, for 1 min. each time                                                                                                                                   | 5 minutes<br>3 x 1 minute                  |
| <b>3. Primary Mouse/Rabbit Antibody</b>            | A. Incubate with Primary Antibody, prepared according to the manufacturer's recommended protocol at the desired concentration. Concentrated Primary Antibodies may be diluted using Primary Antibody Diluent.<br>B. Wash slides with 3 changes of QHD Immuno Wash Buffer. | Refer to antibody data sheet<br>3 x 1 min. |
| <b>4. Quantum HD Polymer Enhancer</b>              | A. Incubate the tissue with Quantum HD Polymer Enhancer reagent.<br>B. Wash slides with 3 changes of QHD Immuno Wash Buffer.                                                                                                                                              | 15 min.<br>3 x 1 min.                      |
| <b>5. Quantum HD HRP</b>                           | A. Incubate the tissue with Quantum HD HRP Polymer-2 reagent.                                                                                                                                                                                                             | 15 min.                                    |



|                                                      |                                                                                                                                                                                                                                                                                                                            |                                                                                                   |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>Polymer -2</b>                                    | B. Wash slides with 3 changes of QHD Immuno Wash Buffer.                                                                                                                                                                                                                                                                   | 3 x 1 min.                                                                                        |
| <b>6. Quantum HD DAB Substrate/Chromogen Mixture</b> | A. Prepare the Stable Quantum HD DAB/substrate working solution by mixing equal parts of Quantum HD DAB Substrate (2x) with Quantum HD Chromogen (2x)<br>B. Monitor level of staining to determine optimal time of incubation.<br>C. Rinse slides with 3 changes of water.                                                 | 5 – 10 min.<br>3 x 1 min.<br>~1 min                                                               |
| <b>7. Counterstain</b>                               | A. Incubate tissue with Quantum HD Hematoxylin Counterstain (or another appropriate counterstain according to manufacturer's recommendation or standard laboratory protocol)<br><br>B. Wash slides with water 3 times, followed by 1 time in QHD Immuno Wash Buffer, then 1 time in water.                                 | 3-5 minutes.<br><br>3 x 1 min. H <sub>2</sub> O<br>1 x 1 min Buffer<br>1 x 1 min H <sub>2</sub> O |
| <b>8. Dehydrate &amp; Coverslip</b>                  | A. Dehydrate tissues through graded ethanol series, followed by xylene series.<br>B. Apply coverslips with permanent mounting medium.                                                                                                                                                                                      |                                                                                                   |
| <b>9. Interpretation of Results</b>                  | A qualified Pathologist is entitled to give a clinical interpretation of the results obtained based on the patient's clinical history and complementary morphological tissue observations. It is the responsibility of the user to identify the best working conditions and the best reagents to perform the staining run. |                                                                                                   |
| <b>Troubleshooting</b>                               | If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact StatLab Medical Products Technical Support at 800-442-3573 or email at <a href="mailto:ihctech@statlab.com">ihctech@statlab.com</a>                                                |                                                                                                   |
| <b>References</b>                                    | Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Supplements 61(01):101, 2012                                                                                                                                                                                                  |                                                                                                   |

